CLAIMS

1. A variant of a parent interferon β (IFNB) polypeptide comprising at least one *in vivo* glycosylation site, wherein an amino acid residue of said parent polypeptide located close to said glycosylation site has been modified to obtain the variant polypeptide having an increased glycosylation as compared to the glycosylation of the parent polypeptide.

2. The variant of claim 1, wherein the glycosylation site is an N-glycosylation site.

3. The variant of claim 1, wherein the parent IFNB polypeptide is a wt IFNB.

4. The variant of claim 3, wherein the wt IFNB is wt human IFNB.

5. The variant of claim 1, wherein the parent IFNB is a variant or fragment of a wt IFNB, which variant or fragment exhibits IFNB activity.

6. The variant of claim 1, wherein the parent IFNB is a variant of a wt IFNB, which as compared to said wt IFNB comprises at least one introduced and/or at least one removed attachment group for a non-polypeptide moiety.

7. The variant of claim 6, wherein the parent IFNB comprises at least one introduced glycosylation site as compared to a wt IFNB.

8. The variant of claim 7, wherein the at least one introduced glycosylation site is an N-glycosylation site.

9. The variant of claim 1 further comprising the mutation C17S relative to the amino acid sequence shown in SEQ ID NO:2.

10. The variant of claim 1, further comprising at least one introduced and/or removed amino acid residue comprising an attachment group for a second non-polypeptide moiety.

11. The variant of claim 10, wherein at least one lysine residue has been introduced and/or removed.

12. The variant of claim 11, comprising at least one mutation selected from the group consisting of K19R, K33R, K45R and K123R.

13. The variant of claim 1, comprising one of the following sets of mutations:
C17S+Q49N+Q51T+F111N+R113T;
C17S+Q49N+ Q51T+D110F+ F111N+ R113T;
C17S+K19R+K33R+K45R+Q49N+ Q51T+D110F+ F111N+ R113T;
S2N+N4T+C17S+Q51N+E53T;
C17S+K19R+K45R+Q49N+Q51T+F111N+R113T+K123R;
C17S+K19R+K33R+K45R+Q49N+Q51T+F111N+R113T+K123R;
S2N+N4T+C17S+K19R+K45R+Q51N+E53T+K123R;
S2N+N4T+C17S+K19R+K33R+K45R+Q51N+E53T+K123R;
S2N+N4T+C17S+K19R+K45R+Q51N+E53T+F111N+R113T+K123R; or
S2N+N4T+C17S+K19R+K33R+K45R+Q51N+E53T+F111N+R113T+K123R

14. The variant of claim 1 comprising the amino acid sequence shown in SEQ ID NO:56 or SEQ ID NO:57.

15. The variant of claim 1, which is glycosylated.

16. The variant of claim 15, which is glycosylated and conjugated to a second non-polypeptide moiety different from a sugar moiety.

17. The variant of claim 16, wherein the second non-polypeptide moiety is a polymer, e.g. PEG, in particular a 12kDa or 20kDa PEG, eg. a single PEG 20kDa.

18. A method of increasing *in vivo* glycosylation of a parent IFNB molecule that comprises at least one *in vivo* glycosylation site, which method comprises

i) substituting an amino acid residue occupying a first position located close to the *in vivo* glycosylation site of the parent IFNB molecule with a second amino acid residue to produce a variant IFNB molecule,

ii) measuring the degree of glycosylation of the variant relative to that of the parent IFNB molecule as obtained from expression in a glycosylating host cell under comparable conditions,

iii) if necessary repeating step i) to substitute the second amino acid residue with a third amino acid residue and/or to substitute an amino acid residue located in a second position close to the glycosylation site with a second amino acid residue and repeating step ii) of either the parent molecule or the variant molecule resulting from step i),

steps i)-iii) being repeated until an increased *in vivo* glycosylation is obtained.

19. The method of claim 18, wherein the glycosylation site is a non-naturally occurring glycosylation site.

20. A variant IFNB molecule obtained by the method of claim 18.

21. The variant of claim 20, wherein the variant comprises a sequence as defined in any of claims 1-14.

22. A variant IFNB molecule which is a fusion protein comprising a) an IFNB polypeptide and b) a human serum albumin polypeptide.

23. A nucleic acid encoding the variant of any of claims 1-14 or 20.

24. An expression vector comprising the nucleic acid of claim 23.

25. A glycosylating host cell comprising the nucleic acid of claim 23.

26. The host cell of claim 25, which is a CHO cell.

27. A method for producing a glycosylated IFNB molecule, which method comprises

i) cultivating the glycosylating host cell of claim 20 under conditions conducive for producing a glycosylated IFNB molecule, and

ii) isolating the resulting glycosylated IFNB molecule.

28. The glycosylated IFNB molecule produced by the method of claim 27.

29. A method for preparing a conjugate, the method comprising reacting the IFNB polypeptide variant of any of claims 1-14, 20 or 22 with the molecule to which it is to be conjugated under conditions conducive for the conjugation to take place, and recovering the conjugate.

30. The conjugate prepared by the method of claim 29.

31. A composition comprising a) the variant of any of claims 1-14, 20 or 22 and b) a pharmaceutically acceptable diluent, carrier or adjuvant.

32. A composition comprising a) the glycosylated IFNB molecule of claim 28 and b) a pharmaceutically acceptable diluent, carrier or adjuvant.

33. A composition comprising a) the conjugate of claim 30 and b) a pharmaceutically acceptable diluent, carrier or adjuvant.

34. A composition comprising a) the nucleic acid of claim 23 and b) a pharmaceutically acceptable diluent, carrier or adjuvant.

35. A composition comprising an IFNB polypeptide that comprises the substitution C17S (relative to SEQ ID NO:2), the composition comprising a reduced amount of stabilizer as compared to the amount required to prepare a pharmaceutical composition comprising an IFNB polypeptide comprising C17.

36. A composition comprising an IFNB polypeptide that comprises the substitution C17S (relative to SEQ ID NO:2), the composition being substantially free from a stabilizer.

37. The variant of any of claims 1-14, 20 or 22 for the treatment of disease.

38. The glycosylated IFNB molecule of claim 28 or the conjugate of claim 30 for the treatment of disease.

39. The nucleic acid of claim 23 for the treatment of disease.

40. The composition of any of claims 32-36 for the treatment of disease.

41. The composition of any of claims 32-36 for the treatment of multiple sclerosis.

42. A method of treating a mammal with a disease for which interferon β is a useful treatment, the method comprising administering to said mammal an effective amount of the composition of any of claims 32-36.

43. The method of claim 42, wherein the disease is multiple sclerosis.

44. A conjugate exhibiting IFNB activity comprising at least one first non-polypeptide moiety conjugated to at least one lysine residue of an IFNB polypeptide, the amino acid sequence of which differs from that of wild-type human IFNB in at least one introduced and/or at least one removed lysine residue.

45. The conjugate of claim 44, wherein the at least one removed amino acid residue is selected from the group consisting of K19, K33, K45, K52 and K123.

46. The conjugate of claim 45, wherein the at least one removed amino acid residue is selected from the group consisting of K19, K33, and K45.

47. The conjugate of claim 44, wherein the at least one removed lysine residue is substituted with an arginine or glutamine residue.

48. The conjugate of claim 31, wherein the interferon β polypeptide comprises one of the following sets of mutations:

K19R+K45R+K123R;
K19Q+K45R+K123R;
K19R+K45Q+K123R;
K19R+K45R+K123Q;
K19Q+K45Q+K123R;
K19R+K45Q+K123Q;
K19Q+K45R+K123Q;
K19Q+K45Q+K123Q;
K45R+K123R;
K45Q+K123R;
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K19Q+K123Q;
K19R+K45R;
K19Q+K45R;
K19R+K45Q;
K19Q+K45Q;
K52R+K134R;
K99R+K136R;
K33R+K105R+K136R;
K52R+K108R+K134R;
K99R+K115R+K136R;
K19R+K33R+K45R+K123R;
K19R+K45R+K52R+K123R;
K19R+K33R+K45R+K52R+K123R; or

K19R+K45R+K52R+K99R+K123R.

49. The conjugate of claim 44, wherein a lysine residue has been introduced in a position selected from the group consisting of N4, F8, L9, R11, S12, F15, Q16, Q18, L20, W22, Q23, G26, R27, L28, E29, Y30, L32, R35, M36, N37, D39, P41, E42, E43, L47, Q48, Q49, T58, Q64, N65, F67, A68, R71, Q72, D73, S75, S76, G78, N80, E81, I83, E85, N86, A89, N90, Y92, H93, H97, T100, L102, E103, L106, E107, E109, D110, F111, R113, G114, L116, M117, L120, H121, R124, G127, R128, L130, H131, E137, Y138, H140, I145, R147, V148, E149, R152, Y155, F156, N158, R159, G162, Y163, R165 and N166 of SEQ ID NO:2.

50. The conjugate of claim 49, wherein the interferon β polypeptide comprises at least one substitution selected from the group consisting of N4K, R11K, G26K, R27K, Q48K, Q49K, R71K, D73K, S75K, E85K, A89K, Y92K, H93K, F111K, R113K, L116K, R124K, G127K and Y155K.

51. The conjugate of claim 50, wherein substitution is selected from the group consisting of Q49K and F111K.

52. The conjugate of claim 44, comprising at least two introduced lysine residues.

53. The conjugate of claim 49, wherein the interferon β polypeptide further comprises at least one removed lysine residue.

54. The conjugate of claim 53, wherein the at least one removed lysine residue is as defined in claim 45, 46, or 47.

55. The conjugate of claim 53, comprising one of the following sets of mutations:
K19R+K45R+F111K+K123R;
K19R+K45R+Q49K+F111K+K123R;
K19R+K45R+Q49K+K123R;

K19R+K45R+ F111K;
K19R+K45R+Q49K+F111K;
K19R+Q49K+K123R;
K19R+Q49K+F111K+K123R;
K45Q+F111K+K123Q;
K45R+Q49K+K123R; or
K45R+Q49K+F111K+K123R.

56. The conjugate of claim 44, wherein the polymer molecule is selected from the group consisting of SS-PEG, NPC-PEG, aldehyd-PEG, mPEG-SPA, PEG-SCM and mPEG-BTC.

57. The conjugate of claim 44, comprising a second non-polypeptide moiety.

58. The conjugate of claim 57, wherein the second non-polypeptide moiety is a sugar moiety, preferably an N-linked sugar moiety.

59. The conjugate of claim 58, wherein the amino acid sequence of the interferon β polypeptide further comprises at least one introduced and/or at least one removed *in vivo* glycosylation site.

60. The conjugate of claim 57, wherein the polypeptide comprises at least one removed amino acid residue comprising an attachment group for the first non-polypeptide moiety, and at least one introduced amino acid residue comprising an attachment group for the second non-polypeptide moiety.

61. The conjugate of claim 60, wherein the amino acid sequence of the interferon β polypeptide comprises at least two removed amino acid residues comprising an attachment group for the first non-polypeptide moiety and at least one introduced amino acid residue comprising an attachment group for the second non-polypeptide moiety.

62. The conjugate of claim 57, wherein the first non-polypeptide moiety is a polymer molecule having lysine as an attachment group.

63. A conjugate exhibiting interferon β activity, comprising at least one polymer molecule and at least one sugar moiety covalently attached to an interferon β polypeptide, the amino acid sequence of which differs from that of wild-type human interferon β in

a) at least one introduced and/or at least one removed amino acid residue comprising an attachment group for the polymer molecule, and

b) at least one introduced and/or at least one removed amino acid residue comprising an attachment group for the sugar moiety,

provided that when the attachment group for the polymer molecule is a cysteine residue, and the sugar moiety is an N-linked sugar moiety, a cysteine residue is not inserted in such a manner that an N-glycosylation site is destroyed.

64. The conjugate of claim 63, wherein the polymer molecule has lysine as an attachment group.

65. The conjugate of claim 64, wherein the polypeptide comprises at least one removed amino acid residue comprising an attachment group for the first non-polypeptide moiety, and at least one introduced amino acid residue comprising an attachment group for the second non-polypeptide moiety.

66. The conjugate of claim 57 or 63, wherein the interferon β polypeptide comprises one of the following sets of mutations:
K19R+K45R+Q49N+Q51T+F111N+R113T+K123R;
K19R+K45R+Q49N+Q51T+F111N+R113T; or
K19R+K45R+Q49N+Q51T+ K123R.

67. The conjugate of claim 44 or 63, wherein the interferon β polypeptide comprises a modified N-terminus that is unavailable for conjugation to a non-polypeptide moiety.

68. A conjugate exhibiting interferon β activity, comprising an interferon β polypeptide comprising a sequence which differs from that of a wild-type human interferon β sequence in at least one introduced glycosylation site, the conjugate further comprising at least one un-PEGylated sugar moiety attached to an introduced glycosylation site.

69. A conjugate exhibiting interferon β activity, comprising an interferon β polypeptide comprising a sequence which differs from that of a wild-type human interferon β sequence in that a glycosylation site has been introduced or removed by way of introduction or removal of amino acid residue(s) constituting a part of a glycosylation site in a position that in the wildtype human interferon β sequence is occupied by a surface exposed amino acid residue.

70. The conjugate of claim 68 or 69, wherein the interferon β polypeptide comprises at least one mutation selected from the group consisting of S2N+N4T, L9N+R11T, R11N, S12N+N14T, F15N+C16S, Q16N+Q18T, K19N+L21T, Q23N+H25T, G26N+L28T, R27N+E29T, L28N+Y30T, D39T, K45N+L47T, Q46N+Q48T, Q48N+F50T, Q49N+Q51T, Q51N+E53T, R71N+D73T, Q72N, D73N, S75N, S76N+G78T, L88T, Y92T, N93N+I95T, L98T, E103N+K105T, E104N+L106T, E107N+E109T, K108N+D110T, D110N, F111N+R113T and L116N.

71. The conjugate of claim 70, wherein the interferon β polypeptide comprises one of the following sets of substitutions: Q49N+Q51T; Q49N+Q51T+F111N+R113T; or Q49N+Q51T+R71N+D73T+F111N+R113T.

72. The conjugate of claim 44, 63, 68, or 69, wherein the interferon β polypeptide further comprises at least one substitution in the position M1, C17, N80 or V101, in particular one of the substitutions M1del, M1K or C17S.

73. A nucleic acid encoding the interferon β polypeptide part of the conjugate of claim 44, 63, 68, or 69.

74. An expression vector comprising the nucleic acid of claim 73.

75. A host cell comprising the nucleic acid of claim 73.

76. The host cell of claim 75, which is a CHO, BHK, HEK293 or SF9 cell.

77. A method of reducing immunogenicity and/or of increasing functional *in vivo* half-life and/or serum half-life of an interferon β polypeptide, the method comprising introducing an amino acid residue constituting an attachment group for a first non-polypeptide moiety into a position exposed at the surface of the protein that does not contain such group and removing an amino acid residue constituting an attachment group for a first non-polypeptide moiety and subjecting the resulting modified polypeptide to conjugation with the first non-polypeptide moiety.

78. The method of claim 77, wherein the non-polypeptide moiety is selected from the group consisting of a polymer molecule, a sugar moiety, a lipophilic group and an organic derivatizing agent.

79. A method for preparing the conjugate of claim 44, 63, 68, or 69, the method comprising reacting the interferon β polypeptide with the molecule to which it is to be conjugated under conditions conducive for the conjugation to take place, and recovering the conjugate.

80. A composition comprising the conjugate of claim 44, 63, 68, or 69 and b) a pharmaceutically acceptable diluent, carrier or adjuvant.

81. The conjugate of claim 44, 63, 68, or 69 for the treatment of disease.

82. The conjugate of claim 44, 63, 68, or 69 for the treatment of multiple sclerosis.

83. A method of treating a mammal with a disease for which interferon β is a useful treatment, the method comprising administering to said mammal an effective amount of the composition of claim 80.

84. The method of claim 83, wherein the disease is multiple sclerosis.

85. A method of treating a mammal having circulating antibodies against interferon β 1a and/or 1b, the method comprising administering to the mammal the composition of claim 80.

86. A cell culture composition comprising a) a host cell transformed with a nucleotide sequence encoding a polypeptide exhibiting interferon β activity and b) medium comprising said polypeptide produced by expression of said nucleotide sequence, said culture composition directly resulting from secretion of said polypeptide from said host cell, and wherein the amount of said polypeptide is at least 800,000 IU/ml of medium, in particular in the range of 800,000-3,500,000 IU/ml medium.

87. The cell culture of claim 86, wherein the host cell is the host cell of claim 75 or 76.